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THE SUSCEPTIBILITY OF GOPHERS, FIELD MICE, AND GROUND SQUIRRELS TO PLAGUE INFECTION.*¹

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THIS investigation was begun with the hope of determining the susceptibility to *B. pestis* of all of the small mammals found in the vicinity of San Francisco, but this was found to be impracticable on account of the difficulty in procuring the necessary animals and in keeping them. Many of the animals obtained died in captivity. It was found, however, that gophers (*Thomomys bottae*), field mice (*Microtus californicus*), and ground squirrels (*Citellus beechyi*) ordinarily stood confinement very well.² We have kept a number of each of these species for several months and they were found to remain in perfect health. Any deaths among these animals will probably occur in the first week of captivity; therefore, to avoid confusion in our work we have used for our experiments only those animals that had been kept in stock for a week or longer. Hares (*Lepus* ———), moles (*Scapanus californicus*), shrews (*Sorex* ———), and weasels (*Putorius xanthogenys*) were frequently captured but they never

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¹ This work was undertaken at the Plague Laboratory of the Public Health and Marine Hospital Service at San Francisco, Cal., at the suggestion of Surgeon M. J. Rosenau, Director of the Hygienic Laboratory, Washington, D. C.

² Stevens, *California Mammals*, San Diego, 1906.

lived long enough to make it practicable to use them for experimental purposes.

CALIFORNIA POCKET GOPHERS (*Thomomys bottae*).

These animals were inoculated by the cutaneous method with tissues from naturally or artificially infected plague rats, as is shown in the following table.

TABLE 1.

Gopher	Materials Used for Inoculation	Day of Death	Day Killed	Lesions	Remarks
No. 1	Spleen natural plague rat No. 64	..	7	None	
No. 2 (small)	Spleen natural plague rat No. 66	3	..	Slight gen. injection	Smears suspicious
No. 3 (small)	Spleen natural plague rat No. 66	..	6	None	
No. 4 (large)	Spleen natural plague rat No. 66	..	10	None	
No. 5 (small)	Spleen of inoculated rat No. 8	4	..	Plague	Liver of this gopher used to inoculate a guinea-pig. The guinea-pig died on the 5th day with typical lesions of plague and a pure culture of <i>B. pestis</i> was isolated from the liver of the latter
No. 6 (large)	Spleen of inoculated rat No. 8	..	8	None	
No. 7 (small)	Spleen of inoculated rat No. 8	..	8	None	

The lesions in the case of No. 5 were an extensive sero-gelatinous exudate at the site of the inoculation and over the whole front of the body; the left inguinal gland was large, hard, and reddish in color, but not caseous. Pest-like organisms were found in the smears made from the sero-gelatinous exudate, the enlarged gland, and from the liver and the spleen. The fact that tissue from this animal conveyed infection to a guinea-pig proved conclusively that the gopher was infected.

It should be mentioned that virulent cultures of *B. pestis* were isolated from the animals that furnished the material for these inoculations: i. e., Rat 64,¹ Rat 66, and inoculated Rat 8.

Of seven gophers inoculated by the cutaneous method, one (No. 5) certainly died of plague; one (No. 2) probably died of plague. It is unfortunate that a guinea-pig was not inoculated from this animal (No. 2), but on account of the limited space available for keeping inoculated animals this was not practicable.

In another experiment, three gophers were inoculated by the subcutaneous method with 0.5 c.c. of an emulsion of the spleen of

¹ Throughout this paper "rat" refers to *Mus norvegicus*.

a guinea-pig dead of acute plague due to inoculation with the spleen from a case of human plague. One of these gophers died within 24 hours after inoculation, showing at the post-mortem examination a reaction at the site of injection; the other two died about 48 hours after inoculation, and presented at necropsy an enlargement of the spleen and an extensive sero-gelatinous edema over the front of the body. From the liver of one of the latter animals a pure culture of *B. pestis* was obtained.

The following was the most convincing experiment in demonstrating the relative immunity of gophers to infection with *B. pestis*. Each of the animals mentioned in the following table was inoculated subcutaneously with one cubic centimeter of suspension in physiological salt-solution of a three-day agar culture of a virulent strain of *B. pestis*. However, the dose of culture suspended in the medium inoculated into the gopher was 100 times as great as that given the rat or the guinea-pig.

TABLE 2.

Animal	Weight of Animal in Gm.	Dose of Culture	Day of Death	Lesions
Guinea-pig ..	285	.0001 loop	4	Acute plague
Rat	280	.0001 "	4	Acute plague
Gopher	135	.01 "	Killed 9th day	None

A culture from the liver of the gopher remained sterile, and a guinea-pig inoculated from the spleen remained healthy.

FIELD MICE (*Microtus californicus*).

These animals appear to be fairly common in the vicinity of San Francisco, as considerable numbers are brought to the laboratory along with the rats from the outlying parts of the city. The average weight of an adult field mouse is about 40 gm.

Experiment 1.—One loopful of a five-day-old virulent agar culture of *B. pestis*, sixth generation, from a plague rat, was used to inoculate each of five field mice by the cutaneous method. The results are shown in the following table:

TABLE 3.

Field Mouse	Day of Death	<i>B. pestis</i> Isolated from Liver
No. 1.....	4	Yes
No. 2.....	4	Yes
No. 3.....	6	No
No. 4.....	Well on 24th day	
No. 5.....	Well on 24th day	

The lesions presented by Nos. 1, 2, and 3 were those mentioned under Exp. 2 (below). Animals No. 4 and No. 5 were alive and well on the 24th day, at which time they were given subcutaneously the same amount of the same culture that proved fatal to the five field mice and the guinea-pig in Experiment 2. Five days later these two animals were chloroformed while they were apparently in perfect health. No lesions were found and smears were negative. I am disposed to believe that these animals were infected by the first (cutaneous) inoculation but recovered and thereby developed an immunity that saved them from the second (subcutaneous) inoculation.

Experiment 2.—Five of the animals were inoculated subcutaneously with a highly virulent culture of *B. pestis* (sixth generation on artificial media) derived from a natural plague rat. Each one was given 1/100 of a loopful of a 24-hour agar culture suspended in physiological salt-solution. One died on the second day (48 hours), three on the third day, and one on the fourth day. The lesions somewhat resembled the lesions of plague in rats. There was a more or less marked general subcutaneous injection and some thickening at the site of inoculation. In only one case was a well-defined bubo present. The spleen was always enlarged and firm. The liver was usually yellowish white instead of the normal brown. A pure culture of *B. pestis* was recovered from the liver of each animal. As a control a guinea-pig weighing 567 gm. was inoculated at the same time with the same dose of the culture. This guinea-pig died on the seventh day with typical lesions of plague.

Experiment 3.—Three field mice were inoculated by the cutaneous method with one loopful of a 24-hour agar culture, first generation, of *B. pestis* isolated from one of the field mice that died on the third day, in the preceding experiment. One died on the third day but as it had been partly mutilated by its companions in the cage, the post-mortem examination was unsatisfactory; however, a general subcutaneous injection was found and I think it may be assumed that this animal died of plague. The other two animals were killed on the 10th day. They presented no lesions. Smears and cultures were negative.

Experiment 4.—The last experiment with field mice was undertaken to determine whether plague infection could be successfully carried through a series of these animals.

1st Passage.—Three field mice were inoculated by the cutaneous method with the spleen from a case of natural rat plague. One died on the third day, presenting the lesions previously noted as due to plague in these animals, and in addition, on the surface of the intestines there were small subserous hemorrhages. The two remaining animals were killed on the eighth day. One of them presented no lesions; the other had an enlarged spleen, and a caseous bubo in one groin in the contents of which were many pest-like organisms (chronic plague).

2d Passage.—The spleen of the first of the preceding animals (dead on third day) was used to inoculate two field mice by the cutaneous method. One of these died on the third day. Cultures from the liver remained sterile but there were the gross lesions usually found in plague in these animals, and in addition whitish granules in the liver. The other died on the fourth day and presented similar lesions at autopsy.

3d Passage.—The spleen of the first of the mice in the second passage was used to inoculate (cutaneous method) another field mouse. This animal died on the third day presenting the usual lesions of plague at necropsy, and had in addition a small, clear, serous, pleural effusion. The experiment had to be discontinued at this point owing to the lack of animals for continuing the series.

GROUND SQUIRRELS (*Citellus beechyi*).

Since this work was undertaken the common ground squirrel of California (*Citellus beechyi*) has been found infected with plague in nature,¹ and several cases of plague in man have been clearly traced to infection in these rodents.

Pioneer work on the experimental infection of these animals was done in 1904 by Passed Assistant Surgeon D. J. Currie, U.S.P.H. and M.H.S., who found them very susceptible to plague infection. Dr. Currie succeeded in infecting these rodents by feeding, by cutaneous and subcutaneous inoculation, and by "contact."

In the course of work now in progress with these animals we have had occasion to inoculate 19 of them by the cutaneous method, and in each case the animal has died of plague within six days of the time of inoculation. The lesions are a large slough at the site of inoculation, a caseous bubo, and an enormously enlarged spleen.

In regard to the cutaneous method of inoculation used in the experiments reported here it is proper to state that the technique was to shave the skin of the abdomen so that a raw surface was presented into which culture or tissue was rubbed. As is well known this method is a very reliable one for infecting animals with *B. pestis*. It practically never fails with guinea-pigs and white rats.

SUMMARY AND CONCLUSIONS.

Gophers are highly resistant to plague when inoculated by the cutaneous method, but apparently often susceptible when inoculated subcutaneously. As only four animals were used for the subcutaneous inoculation, no sure conclusion can be drawn from the experiments.

Field mice are moderately susceptible to cutaneous inoculation and quite susceptible to subcutaneous inoculation. Plague infection was successfully carried directly from animal to animal through three transfers in field mice by the cutaneous method of inoculation.

Ground squirrels are highly susceptible to plague infection, no example of immunity having been encountered.

We may conclude that gophers are not sufficiently susceptible to infection with *B. pestis* to be of any importance from an epidemiological point of view. Field mice are probably about as susceptible

¹ Wherry, *Jour. Infect. Dis.*, 1908, 5, p. 485.

as rats, but as they rarely come in close contact with man, it is improbable that they will ever be a serious factor in the spread of plague. As we already know from experience, ground squirrels are of the utmost importance in plague epidemiology.

The animals used in these experiments represent three families of the order Rodentia, as follows:¹

Citellus beechyi Family Sciuridae

Microtus californicus Family Muridae

Thomomys bottae Family Geomyidae

¹ Elliott, "A Synopsis of the Mammals of North America and the Adjacent Seas," Field Columbian Museum, Chicago, 1901